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LETTER OF ACCEPTANCE

Tuesday, 19 August 2025

Dear authors,

Editor-in-Chief of Narra J has decided that your manuscript has been reviewed and will be published in Narra J as follow:

Authors	: Assyu'ara Al'asyi, Irena Ujianti, Rara Nadika, Zahirah Zahirah, Bimo J. Faizin, Devana A. Afifah, Randhiva Farhan, Salsabila P. Rahmadina, Raisya ZN. Lubis, Sakinah Ghaisani, Kiflan Arkananta, Bety S. Lakshmi and Mulyoto Pangestu
Title	: **Exploring the potential of Holothuria atra extract in modulating fasting triglyceride index and obesity: In silico, in vitro and in vivo studies**
Article Type	: Original Article
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Short Communication

Original Article~~Short Communication~~

Exploring the potential of *H~~h~~olothuria~~oidea~~ atra* extract in modulating fasting triglyceride index and obesity: In silico, in vitro and in vivo studies

Assyu'ara Al'asyi[1], Irena Ujianti[2*], Rara Nadika[1], Zahirah Zahirah[1], Bimo J. Faizin[1], Devana A. Afifah[1], Randhiva Farhan[1], Salsabila P. Rahmadina[1], Raisya ZN. Lubis[1], Sakinah Ghaisani[1], Kiflan Arkananta[1], Bety S. Lakshmi[3] ~~3a a~~nd Mulyoto Pangestu[4]

[1]Medical Program, Faculty of Medicine, Universitas Muhammadiyah Prof. Dr. Hamka, Jakarta, Indonesia; [2]Department of Physiology, Faculty of Medicine, Universitas Muhammadiyah Prof. Dr. Hamka, Jakarta, Indonesia; [3]Department of Public Health, Faculty of Medicine, Universitas Muhammadiyah Prof. Dr. Hamka, Jakarta, Indonesia; [4]Department of Obstetrics and Gynecology, Faculty of Medicine, Nursing and Health Science, Monash University, Clayton, Australia

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Abstract

Obesity remains a global health challenge and is~~,~~ closely associated with metabolic disorders, ~~such as~~ including cardiovascular disease. In response, t~~T~~here is a growing interest in natural interventions that target lipid metabolism to mitigate these risks. Among these, *Holothuria atra* has gained attention for ~~contains~~ its diverse bioactive compounds;~~,~~ ~~but~~ however, its mechanisms of action and therapeutic efficacy ~~require further elucidation~~remain insufficiently understood. The aim of this study was to observe Holothuria atra extract (HAE) and exercise together to affect metabolism to see if this combination better manages obesity by targeting several metabolic processes~~The aim of this study was to…….~~. ~~*Holothuria*~~ *H. atra* specimens were collected from Tablolong Beach, Indonesia, and ethanol extracts were prepared for evaluation. *In silico* study focused on drug-likeness, quantitative structure analysis (QSAR), and network pharmacology. ~~*The extract's phytochemical profile*~~ *of the extract* ~~*was analyzed using liquid chromatography-mass spectrometry (LC-MS), with safety and drug-likeness*~~ *were* ~~*assessed in silico. Network pharmacology methods*~~*approaches* , ~~*including structure-activity relationship (QSAR) modeling and protein target prediction*~~ *were used to* , ~~*explored putative*~~ *potential* ~~*anti-obesity pathways.*~~ *In vitro*, human umbilical cord-derived mesenchymal stem cells (hUC-MSCs) underwent adipogenic differentiation in the presence or absence of the sea cucumber extract. In vivo, male Sprague Dawley rats were assigned to either a control or high-fat diet~~s~~, with subgroups receiving extract supplementation, swimming exercise, or a combination of both for six weeks. Key metabolic parameters were measured biochemically. LC-MS analysis identified 6-gingerol and sarcostin as the principal bioactive compounds~~s~~, both of which fulfilled ~~fulfilling~~ drug-likeness criteria. In silico analyses implicated peroxisome proliferator-activated receptor gamma (PPAR-γ) signaling as a key molecular target. In vitro, extract treatment ~~attenuated~~ was found to attenuate adipogenic differentiation of hUC-MSCs. *In vivo* ~~studies~~ demonstrated ~~that~~ both HAE~~Holothuria atra extract~~ or~~and~~ exercise interventions significantly reduced triglyceride levels, glucose, and the triglyceride index in rats fed a high-fat diet. The combination of HAE~~extract~~ and exercise led to the greatest improvements, with statistical significance in glucose levels (p = 0.000), triglyceride levels (p = 0.007), and the triglyceride index (p = 0.000) compared to the high-fat diet control group. ~~In vivo, both extract and exercise~~ interventions significantly ~~reduced triglycerides~~

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Received: June 2, 2025 - Accepted: Month [X], 2025 - Published online: Month [X], 2025

Short Communication

~~levels, glucose, and the triglyceride index, with the combined intervention treatment demonstrating the greatest improvement (*p*<0.001 vs. high-fat diet group).~~ These findings suggest that ~~*Holothur*~~HAE~~*ia H. atra* extract~~, ~~especially~~ particularly when combined with regular exercise, significantly improves lipid metabolism and holds promise as a complementary approach in obesity management.

Keywords: Obesity, triglyceride index, *Holothuria atra*, sea cucumber extract, lipid metabolism, bioactive compounds~~, in vivo study~~

Introduction

O~~besity has emerged as a pressing public health issue~~b~~,~~ esity has emerged as a pressing public health issue, as evidenced by the global prevalence, which reached 18.5% among adult women and 14.0% among adult men in 2022.(Phelps ~~NH.,~~ *et al.*, 2024) The condition is closely~~closely~~ linked to metabolic conditions such as type 2 diabetes, hypertension, and cardiovascular diseases (Mohajan *et al.*, 2023). Sedentary lifestyles and unhealthy dietary habits significantly contribute to obesity by disrupting lipid metabolism, resulting in elevated fasting triglyceride levels, insulin resistance, and inflammation ~~(Ujianti et al., 2022)~~. These factors highlight the urgent need for natural interventions that can enhance lipid metabolism and mitigate obesity-related health risks. (Ujianti *et al.*, 2022) Sea cucumber extract is a promising candidate for managing triglyceride levels and addressing the complications associated with obesity. This marine organism boasts a rich composition of bioactive compounds, including saponins, chondroitin sulfates, glycosaminoglycans, and fatty acids, which exhibit anti-inflammatory, antioxidant, antidiabetic, and other health-promoting properties (Ujianti *et al.,* 2023). The utilization of sea cucumber extract holds significant biological relevance due to its ability to modulate lipid metabolism, yet the exploration of its specific mechanisms, particularly those involving triterpene glycosides such as sarcostin and their synergy with other bioactive compounds like gingerol, remains limited.(Lu *et al.,* 2024) Addressing this gap represents an opportunity to provide novel insights into this extract's mechanism of action, establishing a unique contribution to the field of lipid metabolism research.

~~Exercise is widely recognized for its metabolic benefits. Regular physical activity enhances lipid metabolism by increasing fat oxidation, reducing triglyceride levels, improving insulin sensitivity, and preventing hepatic fat accumulation (Fitriani *et al.*, 20124). These adaptations reduce the risk of obesity and metabolic syndrome, making physical activity a cornerstone of obesity prevention and treatment. In parallel, sea cucumber extract, (*Holothuria atra*.)~~ has demonstrated the ability to modulate lipid metabolism through mechanisms such as inhibiting pancreatic lipase activity, promoting fat oxidation, and reducing fat synthesis (Shi *et al.*, 2016). Recent studies have shed light on pathways targeted by its bioactive compounds, particularly those involved in lipid metabolism and fat formation (Liu *et al.*, 2023) (Seo *et al.*, 2024). It has also been shown to reduce body weight and fat mass, and to improve insulin sensitivity in animal models (Lu *et al.*, 2022). In paralell, Exercise is widely recognized for its metabolic benefits. Regular physical activity enhances lipid metabolism by increasing fat oxidation, reducing triglyceride levels, improving insulin sensitivity, and preventing hepatic fat accumulation (Fitriani *et al.*, 20124). These adaptations reduce the risk of obesity and metabolic syndrome, making physical activity a cornerstone of obesity prevention and treatment. Given that both interventions influence complementary pathways involved in lipid and glucose metabolism, their combination could potentially exert additive or even synergistic effects. ~~Research reveals its sea cucumber ability to modulate lipid metabolism through mechanisms such as inhibiting pancreatic lipase activity, promoting fat oxidation, and reducing fat synthesis (Shi et al., 2016). Additionally, studies note its role in reducing body weight and fat mass, improving insulin sensitivity, and regulating glucose metabolism in animal models (Lu et al., 2022). Nevertheless, balanced nutrition and physical activity remain critical components of obesity prevention and management (Fitriani et al., 2024). Despite its potential, the mechanisms of action of sea cucumber extract remain incompletely understood. While earlier reports confirm its metabolic benefits, including triglyceride regulation and fat oxidation, gaps in understanding the interplay~~

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Short Communication

~~between specific bioactive molecules (e.g., sarcostin) and pathways such as hepatic lipogenesis, triglyceride storage, and insulin sensitivity suggest fertile ground for novel research contributions. This study uniquely integrates sea cucumber extract's biofunctional effects in combination with exercise, presenting unexplored synergistic mechanisms that amplify its impact on metabolic parameters.~~

~~Recent studies have shed light on pathways targeted by its bioactive compounds, particularly those involved in lipid metabolism and fat formation (Liu et al., 2023) (Seo, H.D., et al. 2024).~~ The aim of this study was to investigate the combined metabolic impact of Holothuria atra extract and exercise, hypothesizing that this integrative approach may enhance the efficacy of obesity management by targeting multiple metabolic mechanisms simultaneously~~The aim of this study was to explore and evaluate the potential of Holothuria atra (sea cucumber) extract in modulating fasting triglyceride index and obesity~~.......... ~~This~~It ~~article study~~ contributes to innovative findings by demonstrating the ability of sea cucumber extract to improve metabolic health by targeting both plasma lipid levels and the triglyceride index, a promising indicator for assessing metabolic risk. Moreover, this study tackles the novelty of combining these bioactives with physical exercise, revealing an amplified, highly effective intervention approach that has not been previously detailed. These findings provide an actionable framework for advancing personalized and integrative therapeutic solutions for obesity management.

Methods

Holothuria atra sampling

Holothuria atra (Jäger, 1957) was identified from a specimen collected by daytime diving in Tablolong Beach Kupang 10°19'07"S 123°27'46", East Nusa Tenggara, Indonesia between May 17 and May 30, 2022~~(Mei 17–30, 2022) as~~ (**Figure 1**~~.~~). Morphological characteristics (elongated, firm body,~~;~~ reddish-black coloration,~~;~~ numerous dorsal papillae,~~;~~ densely packed ventral tube feet) matched published descriptions and aligned with known features of *H. atra*. (Tehranifard, A., Rahimibashar, M~~.~~R., 2012). Taxonomic classification: Animalia; Echinodermata; Holothuroidea; Aspidochirotida; Holothuriidae; *Holothuria*; *H. atra*. This species is commonly found in Indonesian coastal habitats ~~.~~(~~-~~Setyastuti~~, A.,~~ *et al*., 2019). DNA barcoding (*COI* gene) analysis from Tablolong Beach, Kupang, confirmed the identification (99–100% similarity to *H. atra* in NCBI GenBank (Kautsari *et al.*, 2024).

Holothuria atra extraction

The production of sea cucumber extract ~~starts~~ began by removing the body wall from the sea cucumber, which was then ~~and cutting~~ cut it into small ~~pieces~~ segments ~~about~~ approximately 1 cm in size~~, then drying these segments~~and dried. The dried material ~~is then combined~~ was mixed with ethanol ~~in~~ at a 1:5 ratio~~,~~ ~~which~~ to break~~s~~ down cellular proteins and extract~~s~~ secondary metabolites. The mixture was concentrated u~~U~~sing a rotary evaporator (company, city, country) at 40°C~~, the mixture is concentrated~~ through evaporation in 22 hours.

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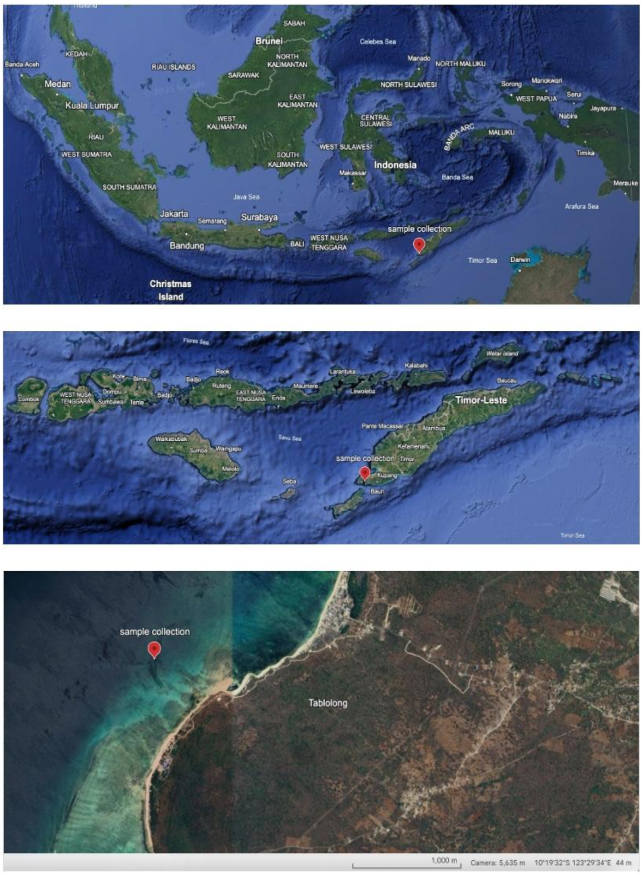

Figure 1. ~~The~~ L~~l~~ocation of the sample collection, Tablolong Beach, East Nusa Tenggara, Indonesia (map pin)~~red circle~~).

In silico methods

Screening of potentially active compounds and toxicity analysis of Sea cucumber

The analysis of bioactive compounds in sea cucumber (~~*Holothuroidea*~~ *H. atra*) extract identified by LC-MS study. According to Lipinski's Rule of Five for shown drug-likeness. These compounds were further analyzed for their molecular properties, such as hydrogen bond donors and acceptors, molecular weight, and LogP values, with ADMETlab 2.0 database to assess their physicochemical characteristics.

Quantitative structure-activity relationship (QSAR) analysis

The analysis of bioactive compounds in *H. atra* for their anti-obesity potential was conducted using the WAY2DRUG PASS prediction system. This tool evaluates compounds by comparing their structural features to those of compounds with known properties through Structure-Activity Relationship analysis. The system's predictions become more reliable when there are stronger structural similarities between the analyzed compounds. This approach is founded on the principle that compounds with similar structures are likely to exhibit similar properties. The system produces a Pa value (Probability to be Active) in 0.4 that indicates how likely a compound

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is to demonstrate specific effects, with higher Pa values suggesting greater confidence in the prediction's accuracy.

Prediction of protein targets and network analysis

Potential of sea cucumber extract targets were identified through the SuperPred database analysis (https://prediction.charite.de/~~URL~~), with only high-confidence results exceeding 80% in both accuracy and probability metrics being considered. The target identification process involved analyzing the previously determined SMILES notation. The DisGeNET database (https://disgenet.com/~~URL~~) was consulted to identify genes and proteins linked to~~o~~ ~~cervical cancer,~~ establishing disease relevance. Further investigation of HAE's ~~teripang's Echinoside B~~ protein targets utilized STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) version 12.0 (https://string-db.org/~~URL~~). The STRING analysis was configured with specific settings: selecting *Homo sapiens* as the studied organism, utilizing the complete STRING network format, and implementing a medium confidence threshold of 0.4. The network data, exported in TSV format from STRING, was then processed using Cytoscape version 3.10.3~~0~~ (Sandiego~~company~~, California~~city~~, United States~~country~~) to create and examine protein interaction networks.

Network analysis employs various centrality measures to elucidate the relationships between target proteins, including Stress, Degree, Betweenness Centrality, and Closeness Centrality. Degree centrality quantifies the number of direct interactions a protein has with other proteins, where a high value indicates a protein with numerous interactions that may serve as a key regulator within the network. Betweenness centrality measures the frequency with which a protein appears on the shortest paths connecting other protein. Closeness centrality reflects the average shortest distance from a given protein to all other protein in the network. Network analysis and enrichment analysis??

In vitro methods

Mesenchym cell culture and treatment

~~The study received approval from the Health Research Ethics Committee of the Faculty of Medicine, University Muhammadiyah Prof.Dr.Hamka, under the number KEPKK/FK/071/17/2022.~~ Mesenchymal stem cells (MSCs) from h~~H~~uman umbilical cord (hUC) were cultured in 12 culture well plate ~~12~~ using Minimum Essential Medium Eagle - Alpha Modification (Alpha MEM) with Nucleosides~~alpha minimum essential (Amem)~~ ~~medium~~ supplemented with 10% Platelet Rich Plasma (PRP). The cells were incubated at a temperature of 37 °C with 5% CO2. The culture medium was replaced every 2-3 days to ensure optimal cell growth.

Before any interventions were performed, a standardized protocol was followed for subculturing the cells. This involved rinsing the cells with phosphate-buffered saline (PBS), followed by a 2-minute incubation with 1 mL of 0.05% trypsin-EDTA. The cells were then transferred to a 15 mL tube containing 10 mL of Amem medium supplemented with 10% PRP and centrifuged at a speed of 1200 rpm for 10 minutes at a temperature of 20°C.(Pawitan et al., 2015) The supernatant was discarded, and the cell pellet was resuspended in 1 mL of medium. Cell viability was assessed using Tryphan Blue assay. Subsequently, the cells were seeded in a culture well plate 12 at a density of 5000 cells/cm2 per well and incubated at 37 °C with 5% CO2. The adipogenesis medium induction was performed and replaced every 2-3 days. The induction of adipogenesis was done concurrently with the application of sea cucumber extract at concentrations of 9 ûgr/ml and 19 ûgr/ml

In vivo methods

Experimental a~~A~~nimals ~~and categorization~~

Ten-week-old healthy male Sprague Dawley rats were obtained from Institut Pertanian Bogor (IPB). The animals underwent a one-week acclimatization period in the Central Animal Lab at the Department of Chemistry, Universitas~~y~~ ~~of~~ Indonesia, where environmental conditions were maintained at 24°C with 50% humidity. After acclimatization, the rats were randomly divided

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into five experimental groups of 5 rats each: control diet group, high-fat diet group, high-fat diet plus extract group, high-fat diet with exercise group, and high-fat diet with both extract and exercise group. The rats were housed individually throughout the study period, with environmental conditions maintained between 18-26°C and 50% humidity. They were fed twice daily and had free access to water, which was replaced every two days. The researchers monitored each rat's weight, size, and waist measurements monthly. Blood tests measuring serum lipids, glucose, and insulin levels were performed at the study's conclusion.

Animal nutrition+extract

Laboratory rat feed was sourced from Biorat Laboratory Animal Co., Ltd., including both standard and specialized high-fat, high-salt diets. The standard feed contained 10% fat, 22% protein, 68% carbohydrates, and 0.5% salt. The specialized high-fat diet had substantially higher levels of fat and salt, containing 49% fat, 21% protein, 30% carbohydrates, and 2% salt. Rats in both the HFD-extract and HFD-extract-exercise groups were fed the high-fat diet and received daily extract supplements of 300 mg per kilogram of body weight via gastric administration.

Exercise Protocol

Swimming training was conducted in a barrel filled with water at 33–35°C to a depth of 40–50 cm, allowing the rats to swim freely. The first session lasted 15 minutes, and the duration increased by 5 minutes daily until 30 minutes. Rats in the HFD+Exercise and HFD+Exercise+extract groups swam for 30 minutes daily, 5 days a week, for 6 weeks, while those in the other groups remained sedentary in cages (Lu et al., 2016)

Measurement of fasting glucose, fasting triglyceride, and triglyceride index

Each rat's body weight, body size, and abdominal circumference were measured before and after treatment. Abdominal circumference (AC) was assessed on the largest zone of the rat abdomen using a plastic non-extensible measuring tape, and body size was defined as the length from nose to anus. Lee's obesity index was calculated using the formula: Lee's index = cube root of body weight (g)/body size (cm). ~~Rats were euthanized in lethal dose by administering ketamine 90 mg/kg and xylazine 10 mg/kg with diluted sterile saline. The anesthetic mixture was injected intraperitoneally. Rats were placed individually in clean cages and observed for euthanasia onset. (Oh *et al.*, 2024)~~ Following a 12-hour fast, blood samples were collected from the orbital sinus of rats to measure ~~Bbefore and After treatments the rats were takes sacrificed, aorta tissues were sampled for homogenate tissue and Hematoxylin-Eosin (HE) staining. After a 12-hour fast, blood samples for measurewere collected from rat tails, and levels of~~ fasting blood glucose (FBG) with a threshold of 90 mg/dL and ~~,~~ triglycerides (TG) with a threshold of 120 mg/dL.~~,~~ ~~high-density lipoprotein (HDL), and low-density lipoprotein (LDL) were measured~~These measurements were conducted before and after treatment ~~every 8 weeks~~ using a Hitachi 7600 automatic biochemical analyzer (Hitachi; Tokyo, Japan). The Triglyceride-Glucose (TyG) Index by comparing between triglyceride and glucose levels. The Triglyceride-Glucose (TyG) Index is indeed used as a surrogate marker for insulin resistance. Some studies have proposed that TyG Index < 8.3: Low likelihood of insulin resistance TyG Index 8.3 - 8.9: Moderate likelihood of insulin resistance TyG Index > 8.9: High likelihood of insulin resistance.(Delgadillo *et al.*, 2024) Rats were euthanized using a lethal dose of ketamine (90 mg/kg) and xylazine (10 mg/kg) diluted in sterile saline. The anesthetic mixture was injected intraperitoneally. After administration, rats were placed individually in clean cages and observed until euthanasia occurred. (Oh *et al.*, 2024)

Statistical analysis

For *in vivo* study Statistical results are presented as means ± standard deviations, with experimental groups containing 5-7 animals each. The specific number of animals used in each experiment is indicated in the corresponding figure legends. We performed one-way ANOVA to assess differences between groups, followed by Tukey's post-hoc test for multiple comparisons. Results were considered statistically significant when *p*~~P~~<0.05. All statistical analyses were conducted using SPSS version 20.0 software (IBM~~company~~, Chicago~~city~~, USA~~country~~).

Results

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Phytochemical study and toxicity analysis

~~Phytochemical analysis was performed to evaluate the most bioactive compounds in the extract. Using~~ LC~~-~~MS study showed ~~in **Figure 2**~~ that 6-Gingerol and sarcostin ~~is~~ was the most bioactive compounds ~~that~~ contained in the extract (**Figure 2**~~.~~). Sarcostin demonstrated 176,739 with consistent RMS metrics, recorded at a retention time of 18.39 minutes. 6-Gingerol showed an even greater response value of 331,644 at a retention time of 18.11 minutes, indicating its substantial contribution to biological activity.

Table 2~~1~~ and **Figure 3** showed that 6-Gingerol and Sarcostin have significant potential as drug-like molecules, fulfilling Lipinski's Rule of Five.(Karami *et al.*, 2022) ~~-~~6-Gingerol has a molecular weight of 294.39 g/mol, a LogP value of 3.48, 2 hydrogen bond donors, and 4 hydrogen bond acceptors. ~~exhibits properties that~~These values support stability, solubility, membrane permeability, structural flexibility, and moderate lipophilicity~~stability, solubility, and membrane permeability, along with structural flexibility and moderate lipophilicity~~. Sarcostin, possesses a molecular weight of 382.5 g/mol, a LogP value of 2.35, 6 hydrogen bond donors, and acceptors, indicating higher hydrophilicity; although its greater number of hydrogen bond donors and acceptors could influence membrane permeability.~~in contrast, displays higher hydrophilicity although its greater hydrogen bond count could influence membrane permeability~~

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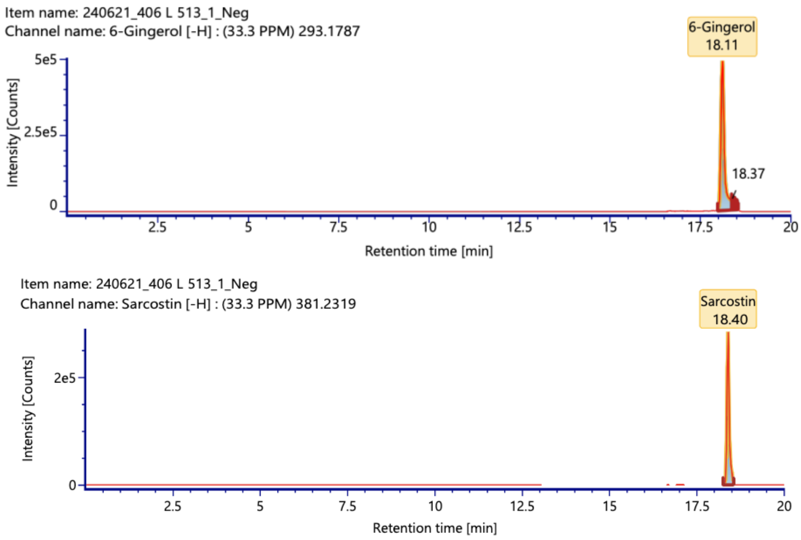

Figure 2. LC-MS analysis of seacucumber extract. Mass spectrum for the sea cucumber extract showing signal intensity in relation to molecular weight. The peaks in the graph represent the detected compounds.

Table 1. **Identification and Analytical Parameters of Compounds Detected by LC-MS Study**

Component name	**Observed RT (min)**	**Fragments Found**	**Match Mz RMS PPM**	**Intensity RMS Percent**	**Response**
3-O-(Z,E) 4,2-Decadienoyl ingenol	16.62	60	4.81	19.15	2031
6-Gingerol	18.11	13	13.15	16.19	353041
Andropanideol	17.81	1	8.38	25.11	15350
Cimifoside IV	16.65	143	21.64	114.91	1119
Ganoderic acid H	16.58	24	8.22	15.89	2769
Hederaagenin 3-O-α-L-rhamnopyranosyl-	16.58	76	6.96	81.31	1059
Isolidylfutoquicinol B	17.51	1	5.98	48.24	1051
Kukoamine A	18.17	2	6.06	16.18	15455
Mulberrofururan D	16.64	8	6.00	824.89	3315
Nigalatacine J	17.88	3	13.39	80.94	74070
Nobilin C	17.74	0	2.82	15.73	1125
Ostruthin	18.16	1	12.20	16.55	121610

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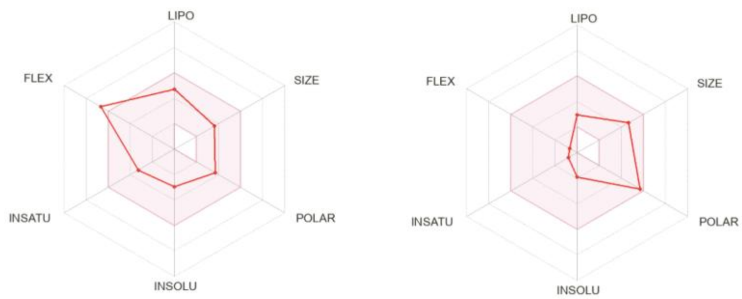
Component name	Observed RT (min)	Fragments Found	Match Mz RMS PPM	Intensity RMS Percent	Response
Paeonolide G	16.62	68	4.97	44.83	9365
Sarcostin	18.40	1	12.18	12.96	196319
β-Cryptoxanthin	18.48	17	4.99	15.50	31519

A total of 15 compounds were successfully identified in the [sample/plant extract] using UPLC-QTOF-MS analysis, as summarized in Table 1. The compounds were annotated based on their accurate masses, retention times (RT), fragmentation patterns, and isotope match scores. Retention times for the identified compounds ranged from 16.58 to 18.48 minutes. All compounds exhibited mass errors within an acceptable range (−8.7 to 9.6 ppm), supporting the reliability of the measured masses for tentative compound identification. Among the detected compounds, 6-Gingerol showed the highest response, with an intensity of 353,041, indicating its abundance in the sample. Other prominent compounds included Sarcostin ~~(response: 196,319)~~, Ostruthin ~~(response: 121,610)~~, and Nigalatacine J (resp~~onse: 74,070~~). On the other hand, several compounds, such as Cimifoside IV and Hederaagenin 3-O-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-α-L-arabinopyranoside, were present at lower concentrations (responses: 1,119 and 1,059, respectively). The number of fragments found varied considerably, from 0 (Nobilin C) to as many as 143 (Cimifoside IV), indicating differences in fragmentation behavior and possibly molecular structure complexity. Additionally, isotope match scores for both m/z and intensity RMS percent further corroborated the identity assignments, with most compounds exhibiting low RMS values, reflecting good matches to theoretical isotope distributions. All detected compounds were observed as $[M-H]^-$ adducts, consistent with negative mode ionization typically used for this class of metabolites. The wide range of detected molecules included diterpenoids, glycosides, and other specialized metabolites, suggesting chemical diversity in the analyzed sample. These results collectively provide a comprehensive chemical profile of the [sample/plant extract], supporting further quantitative or bioactivity-guided investigations.

Table 2~~1~~. ~~The~~ Toxicity ~~a~~Analysis of ~~most Bioactivecompound~~ 6-gingerol and sarcostin from *Holothuria atra* extract

Molecule type	Hydrogen bond donors (HBD)	Hydrogen bond acceptors (HBA)	Molecular weight (MW)	Log P
6-Gingerol	2	4	294.4 g/mol	3.48
Sarcostin	6	6	382.5 g/mol	2.35



Short Communication

Figure 3. The toxicity analysis of most bioactivecompound holothuria atra extract

Quantitative structure-activity relationship (QSAR) analysis

~~Based on SAR analysis using Way2Drug Pass Online, bioactive compounds in sea cucumbers were found to have good potential for various applications related to metabolic disease prevention.~~ Based on structure–activity relationship (SAR) analysis using Way2Drug PASS Online, bioactive compounds identified in sea cucumbers demonstrated promising pharmacological potential, particularly for metabolic disease prevention. According to PASS Online criteria, a PA (probability to be active) score greater than 0.3 indicates significant pharmacological activity. Compounds in sea cucumbers also show structural similarity to some drugs, such as diabetes drugs (PA Score 0.405), and drugs for dyslipidemia (PA Score 0.328), which can also act as candidat obesity drugs, as shown in **Figure 4**.

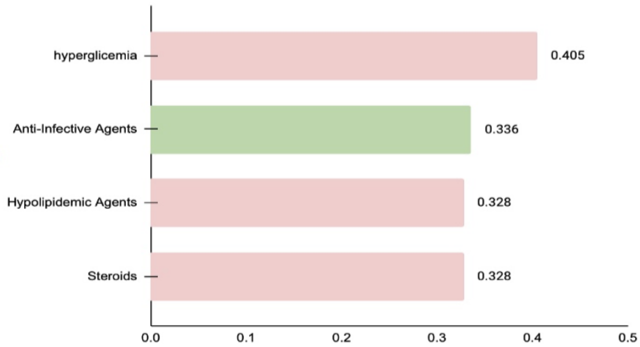


Figure 4. Structure-activity relationship (SAR)-based prediction of sea cucumber-derived bioactive compounds for metabolic disease applications. The figure illustrates SAR analysis results using Way2Drug PASS Online for bioactive compounds found in sea cucumbers. ~~Association strengths between specific drug classes/adverse events and the outcome of interest.~~ X-axis represents ~~Odds Ratio (OR)~~Potential activity values, while the Y-axis lists the drug classes/adverse events. Red bars indicate ~~increased risk (OR > 1)~~metabolic activity, green bar ~~indicates decreased risk (OR < 1).~~

Network analysis

PPAR-γ is considered a potential target because it has the highest values in terms of betweenness centrality, closeness centrality, degree~~, and cervical cancer overall~~ score compared to other targets (**Table 2** and **Figure 5**). Based on these analyses, it is predicted that *H.atra*, specifically through the PPAR-γ pathway, may be effective in targeting obesity, dyslipidemia, and insulin resistance. Of these targets, PPAR-γ exhibits the greatest number of connections within the network, with 23 direct interactions, highlighting its pivotal function as a central hub.

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Commented [A56]: Cervical cancer score is irrelevant to a diabetes-focused study. Remove or justify.

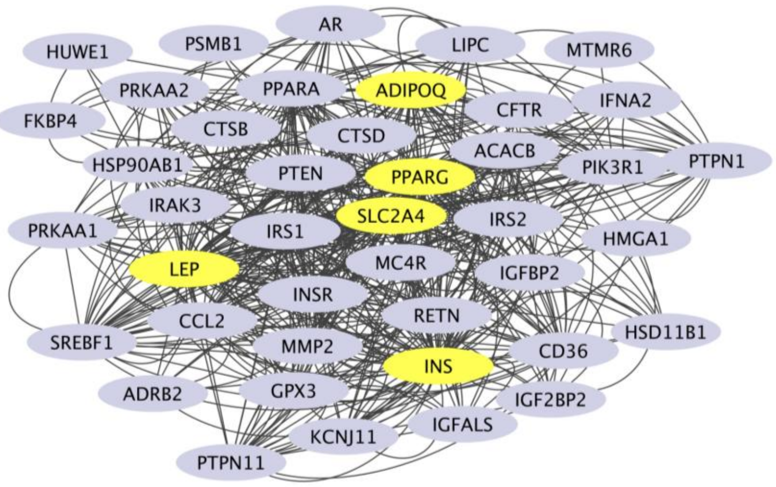

Figure 5. Network analysis for sea cucumber Target. The figure depicts the network analysis highlighting key targets associated with the biological activity of Sea Cucumber. ~~Among these targets, PPAR-γ, INS, LEP, SLC2A4 and ADIPOQ demonstrates the highest values for betweenness centrality, closeness centrality, and degree, as well as the highest cervical cancer overall score~~. Among these, PPAR-γ demonstrates the highest degree,23 interactions, with other nodes, underscoring its central role.

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Table 2. ~~Betweenness centrality score of N~~Nnetwork analysis results.

Name	Degree	Betweenness centrality	Closeness centrality
PPARG	66	0.15	0.82
LEP	52	0.05	0.73
SLC2A4	44	0.02	0.68
ADIPOQ	42	0.02	0.64
INS	68	0.17	0.85
PPARA	44	0.06	0.68
SREBF1	40	0.03	0.65
INSR	38	0.03	0.65
IRS1	42	0.03	0.67
PTEN	42	0.08	0.67

In vitro result

In vitro differentiation

At the outset, the HU-MSCs show a customary fibroblast-like morphology, featuring small cell bodies with elongated cell processes across all treatment groups. Upon reaching the 5th passage, during the commencement of the treatment, control group cells demonstrated differentiation towards mature adipocytes. This resulted in cells adopting a spherical shape, their cytoplasm riddled with large fat droplets in preparation for forming a single lipid droplet. Control group cells without differentiation retained the fibroblast-like morphology typical of MSCs at the beginning of the treatment. In contrast, the sea cucumber extract treatment group elucidated a fibroblast-like structure but showed the presence of a cytoplasm filled with minimal fat vacuoles. The treatment group given a 9 ug/ml extract dose displayed larger vacuoles than the group treated with a concentration of 19 ug/ml, showed in **Figure 6**.

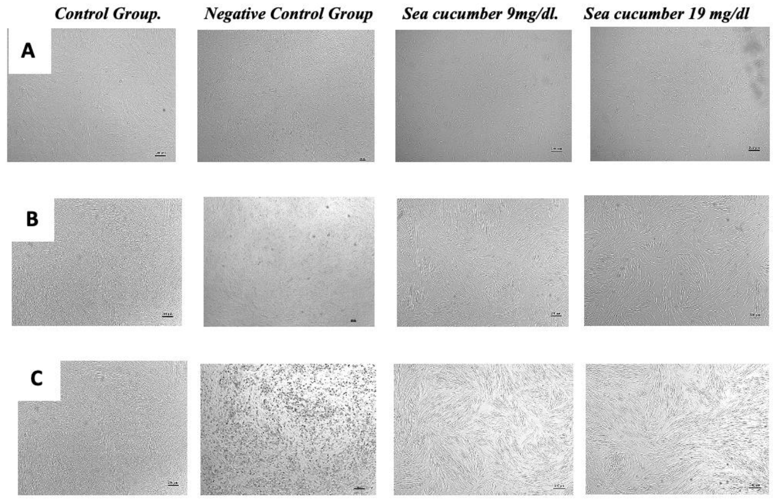

Figure 6. MSCs from positive control group, negative control group and treatmen group (9 mg/dL and 19 mg/dL). Representative differentiation of cells from passage 5 in 7 days (A), 9 days (B) and 12 days (C).

In vivo results

In vivo biochemical results

Based on the data in the table, the administration of extracts and exercise, either separately or together, can improve the disturbed biochemical parameters caused by a high-fat diet. This includes reducing triglycerides, glucose, triglyceride index, and weight gain. The combination of extract administration and exercise in the HFD + Extract + Exercise group showed the most optimal results in improving these parameters as shown in **Table 3**.

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Table 3. Effect of sea cucumber extract on biochemical parameters

Parameter (pasma serum)	Normal diet	High fat diet (HFD)	HFD+extract	HFD+exercise	HFD+extract +exercise
Triglyceride (mg/dL)	110.2±10.6	143.2±7.72	100.2±10.2**	97.0±5.50**	66.0±0.77***
Glucose (mg/dL)	75.6±4.41	100.8±1.68	89.0±3.30	90.8±2.08	84.21±0.29**
Triglyceride index	8.30±0.11	8.9±0.05	8.4±0.09**	8.4±0.05**	8.0±0.05***

The effect of sea cucumber extract on animal and biochemical parameters. The data is presented as mean±SD (n=5 per group). The statistical differences were revealed by one-way ANOVA and Tukey's post hoc test. The mean differences were significant compared with the High Fat Diet group.

*Statistically significant at $p< 0.05$

**Statistically significant at $p<0.01$

***Statistically significant at $p<0.001$

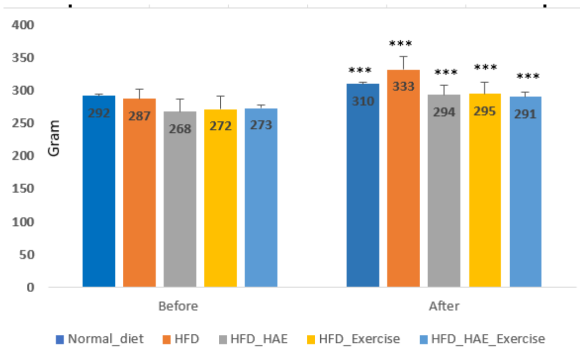

Figure 7~~6~~. Body weight (grams) of experimental groups before and after intervention. Mice were divided into five groups: Normal diet, high-fat diet (HFD), HFD with sea cucumber extract (HFD_HAE), HFD with exercise (HFD_Exercise), and HFD with sea cucumber extract plus exercise (HFD_HAE_Exercise). Data are presented as mean ± SD. Significant differences between the HFD group and all other groups after intervention are indicated (***$p < 0.001$).~~MSCs from positive control group, negative control group and treatmen group (9 mg/dL and 19 mg/dL). Representative differentiation of cells from passage 5 in 7 days (A),. 9 days (B) and 12 days (C).~~

Discussion

The bioactive compound from sea cucumbers, has shown significant potential as a natural treatment for obesity and related metabolic complications. In silico studies provide foundational insights into how sea cucumber compounds interact with genes associated with obesity. Investigations by Hoang and Amir et al. revealed that bioactive compounds from sea cucumber modulates metabolic and inflammatory pathways. It increases adiponectin (ADIPOQ) levels, enhances insulin sensitivity, and suppresses pro-inflammatory cytokines like TNF-α (Jayaraman et al., 2021). Moreover, the compound activates PPAR-γ, a critical regulator of lipid metabolism (Zhang et al., 2024). It also restores the activity of SLC2A4/GLUT4, improves glucose uptake, and reduces insulin resistance (Pérez-García et al., 2022). These mechanisms collectively enhance insulin receptor function and support effective glucose metabolism, preventing metabolic disorders (Shi et al., 2016; 2019). Further in vitro research has validated these molecular mechanisms (Liu et al., 2023). Studies have shown that administering sea cucumber extracts reduces NFκB levels, directly inhibiting fat cell production or adipogenesis (Al Madhoun et al., 2023). AAmir et al. demonstrated that NFκB regulates PPAR-γ expression, which is essential for adipocyte differentiation, highlighting the extract's ability to control fat accumulation (Aamir et al., 2020). Additionally, the extracts inhibit lipid storage regulators like fatty acid synthase (FAS) and the NFκB/IKK signaling pathway (Jayaraman et al., 2021). These results align with in vitro findings by Parida et al., which indicated a reduction in lipid accumulation markers, including TNF-α and NFκB (Parida et al., 2019). Overall, the anti-adipogenic and anti-inflammatory effects of sea cucumber extracts, as demonstrated in vitro, show promise for managing obesity.

In vivo studies further build on these findings, highlighting the physiological effects of consuming sea cucumber extracts in animal models of obesity. In mice fed a high-fat diet (HFD), those treated with sea cucumber extracts experienced significant weight loss compared to the control group (Li et al., 2021; Chen et al., 2018). Gingerol, a prominent bioactive compound in the extract, is known to enhance lipid metabolism by increasing lipolysis and thermogenesis while also inhibiting lipogenesis. (Lee et al., 2021) Its anti-inflammatory and antioxidative properties further promote metabolic health by reducing systemic inflammation and oxidative stress, both of which are closely linked to obesity and insulin resistance.(Adegbola et al., 2024) Sarcostin , a secondary metabolite known as triterpene glycoside, hypothesized to contribute synergistically through its potential to modulate lipid metabolism and inhibit intestinal fat absorption. (Li et al.,

2021) This dual action of the sea cucumber extract, along with its ability to improve insulin sensitivity, may account for its efficacy in controlling triglyceride levels and triglyceride index, key indicators of metabolic risk. Otherside, physical activity also plays an essential role in improving metabolic health by increasing adiponectin, PPAR-γ, and GLUT4 levels, which enhance insulin sensitivity and promote glucose and lipid metabolism (Polito et al., 2020). Exercise complements the effects of sea cucumber extracts by reducing leptin levels, supporting appetite control, and improving blood lipid profiles through increased PPAR-α activation (Lu et al., 2016). When combined with sea cucumber extracts, physical activity optimizes insulin signaling pathways, enhances fat breakdown, and reduces inflammatory markers. This dual approach offers protection against oxidative stress, leading to better weight management, glucose uptake, and overall metabolic regulation (Pérez-García et al., 2022). Hen studies showed that with C57/BL6 mice fed a high-fat diet and supplemented with sea cucumber saponins and phospholipids enriched with eicosapentaenoic acid improved glucose tolerance and insulin sensitivity (Han et al., 2019). Another study with liposomal formulations of sea cucumber saponins proved highly effective in reducing obesity and cholesterol levels in mouse models (Chen et al., 2018). These results align with prior research and highlight the compound's ability to regulate glucose and lipid metabolism (Jin et al., 2018). It targets obesity-related pathways, offering a holistic approach to addressing obesity and its related metabolic dysfunctions (Jin et al., 2018). These findings are further supported by several studies (Kalinin et al., 2020; Kang et al., 2015; Shi et al., 2016).

~~In conclusion, ~~E~~e~~vidence from in silico, in vitro, and in vivo studies provides solid insights into the anti-obesity potential of sea cucumber-derived compounds, particularly ~~flavonoid~~ 6-gingerol and sarcostin as terpenoid~~triterpenoid~~. ~~Flavonoids and triterpenoids~~6-g~~G~~ingerol and sarcostin are the two most potent bioactive compounds in inhibiting adipogenesis via the PPARγ pathway. While terpenoids possess additional effects—such as regulating the AMPK pathway and exhibiting antioxidant properties—they are considered particularly specific in targeting PPARγ, as they can disrupt its ligand-binding activity. ~~While flavonoids exhibit additional effects, such as regulating the AMPK pathway and demonstrating antioxidant properties, triterpenoids are considered more specific and directly target PPARγ by disrupting its ligand-binding activity~~ (~~[~~Germoush et al., 2019) (Grace et al., 2019)~~e~~~~XX]~~. Therefore, when considering direct influence on PPARγ, triterpenoids are likely to have the greatest impact. By modulating adipogenesis, fat production, and glucose metabolism while reducing systemic inflammation, sea cucumber extracts emerge as a promising natural therapy for obesity and related metabolic disorders (Hosseini et al., 2022). When combined with lifestyle changes like physical activity, the therapeutic effects of this compound are enhanced, offering a comprehensive strategy for managing obesity and improving metabolic health (Li et al., 2021). Future efforts should focus on enhancing its bioavailability, isolating and characterizing specific bioactive components, exploring underlying molecular pathways, and conducting clinical trials to confirm its therapeutic efficacy. These findings highlight the clinical potential of sea cucumber extract as a natural alternative for managing obesity.

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The limitations of this study that the findings are primarily based on preclinical models and male Sprague Dawley rats, so further research involving human clinical trials and a wider range of animal subjects is necessary to confirm the translational relevance and to address potential sex-related differences. ~~..~~

Conclusion

This study demonstrates that ~~*Holothuroidea*~~ *H. atra* extract, significant anti-obesity and lipid-modulating effects through inhibition of adipogenesis, reduction of systemic inflammation, and improvement of metabolic parameters, especially when combined with exercise. While these findings support its potential as a natural multi-target intervention for obesity and its related disorders, further investigation is necessary to isolate individual active compounds, thoroughly elucidate molecular mechanisms, determine pharmacokinetic properties, assess long-term safety, and confirm efficacy through rigorous clinical trials.

Ethics approval

The research protocol was approved by the ethics committee of Universitas Muhammadiyah Prof. Dr. HAMKA under permit number KEPKK/FK/058/05/2024.

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Competing interests

All the authors declare that there are no conflicts of interest.

Funding

This study received no external funding.

Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

Declaration of artificial intelligence use

~~If authors used the AI during the study or manuscript preparation, please add the statements (chose one or more the alternative) and add compulsory statement.~~

~~*"This study used artificial intelligence (AI) tools and methodologies in the following capacities* (**CHOOSE & EDIT AS APPROPRIATE**):~~

1. ~~**Data analysis and modeling**: Machine learning algorithms, including [specific algorithms or techniques], were used to analyze the dataset and predict outcomes. These were implemented using [software/tools, e.g., Python, Scikit-learn, TensorFlow].~~
2. ~~**Data preprocessing**: AI-assisted techniques [state the technique(es)] were/was applied for data cleaning, feature selection, and transformation to prepare the dataset for analysis.~~
3. ~~**Visualization**: AI tools, such as [e.g., Tableau, Matplotlib with AI extensions], were/was used for generating graphs, charts, and visual summaries.~~
4. ~~**Manuscript writing support**: AI-based language models, such as [e.g., ChatGPT, Quillbot], were/was employed to (**CHOOSE AS APPROPRIATE**):~~
    a. ~~Language refinement (improving grammar, sentence structure, and readability of the manuscript).~~
    b. ~~Content summarization (assisting in summarizing findings and conclusions in a concise manner).~~
    c. ~~Technical writing assistance (providing suggestions for structuring complex technical descriptions more effectively)~~
    d. ~~Generate scientific content, interpret data, and draw conclusions~~
5. ~~**Simulation and forecasting**: Predictive modelling and simulations were conducted using AI frameworks to validate research hypotheses.~~

~~**PLUS**~~

~~*"We confirm that all AI-assisted processes were critically reviewed by the authors to ensure the integrity and reliability of the results. The final decisions and interpretations presented in this article were solely made by the authors."*~~

~~This declaration does not apply to the use of basic tools for checking grammar and spelling (such Grammarly), references (EndNote, Zotero, etc), SPSS.~~

~~If there is nothing to disclose:~~

We hereby confirm that no artificial intelligence (AI) tools or methodologies were utilized at any stage of this study, including during data collection, analysis, visualization, or manuscript preparation. All work presented in this study was conducted manually by the authors without the assistance of AI-based tools or systems.

How to cite

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